Claim 18 (New). A plant according to claim 8 or claim 10 in which said plant is resistant to aflatoxin.

Claim 19 (New). A plant according to claim 8 or claim 10 in which said plant is resistant to corn borer.

Claim 20 (New). A plant according to claim 8 or 10, further comprising a novel band identified by the SSR probe bnlg1805 thereof,

in which the roots of said plant have aerenchyma.

Claim 21 (New). A plant according to claim 8 or 10, further comprising one or more novel bands identified by SSR probes dupSSR23, phi123, bnlg2235 or bnlg1714 thereof,

in which said plant has tolerance to corn rootworm.

Claim 22 (New). A plant according to claim 8 or claim 10, in which said plant has tolerance to low nitrogen.

**Nonstatutory Double Patenting**

The Examiner has rejected the claims 2-6, 8-12 and 14 as obviousness-type double patenting based on claims 1-9 and 11 of Eubanks US Patent No. 5,750,828, 1998, in which claim 1 describes a method of crossing a *Tripsacum dactyloides* plant and a *Zea diploperennis* plant and harvesting seed from the hybrid plant, and claim 6 describes a method of crossing a *Tripsacum dactyloides-Zea diploperennis* hybrid plant with *Zea mays* and harvesting the seed. Claims 2-5 are dependent on claim 1, and claims 7-9 and 11 are dependent on claim 6. The Examiner further asserts that the restriction fragments as claimed in claim 2 of this application

would occur during any form of mitotic or meiotic cell division and replication in the plant cell cycle, and thus, would not make the instant claims non-obvious over Eubanks claims 2-6, 8-12 and 14 of US Patent No. 5,750,828 1998. However, the applicant respectfully submits that the presence of one or more of the novel restriction fragments claimed in claim 2 distinguishes the plants and materials claimed in claims 2-6, 8-12 and 14 as non-obvious because they mark the presence of mutant alleles that would not have been expected or predicted to occur in plants obtained by making crosses as described in the methods of Eubanks US Patent No. 5,750,828 1998. It is common scientific knowledge that mutations are extremely rare events, occur at low frequencies, and do not occur in clusters. The novel restrictions fragments in claim 2 identify precise mutations that occur in high frequencies and clusters in recombinant *Tripsacum*-teosinte progeny from different *Tripsacum* and teosinte parents, and are therefore completely unexpected and non-obvious, as discussed below and in the specification,

Basic genetic principles state that the offspring of two parents exhibit a combination of restriction fragments from both parents and can be identified by comparing their DNA fingerprints to those of the parents. As described on page 19 of the specification, the offspring of two parents are expected to inherit an allele for a genetic locus from each parent. If both parents have the same allele for a gene, the offspring are expected to inherit the same restriction length fragment polymorphism (RFLP as defined on page 17 of the specification, i.e. allele as defined on pages 15 of the specification) from both parents. If each parent carries a different allele at the same locus, the offspring will inherit a different polymorphism from each parent. Because the heritability of RFLPs is highly stable, reliable, and predictable in plants, RFLP genotyping is a DNA fingerprinting method routinely used to determine paternity in plants. On rare occasions an individual offspring may exhibit an RFLP not present in either parent. The novel RFLP

signals a mutation has occurred and the progeny now carries a new, mutant allele not found in either parent. However, as described on page 20 of the specification, such mutations are rare changes in the genetic material that are usually harmful. Mutation rates range from 1 in 1,000 to 1 in 1,000,000 gametes per generation. Therefore, siblings that have the same parents would not be expected to share a common mutant allele (i.e. RFLP), and cluster of mutations do not occur within families. Mutations are extremely rare, occur in low frequencies, and do not occur in clusters. Therefore, it would not be expected by anyone skilled in the art re: claim1 in Eubanks US Patent No. 5,750,828 1998 that any of the various (*Tripsacum dactyloides* X *Zea diploperennis*) or (*Zea diploperennis* X *Tripsacum dactyloides*) plants described would exhibit the same novel restriction length fragment polymorphisms for each of the respective RFLP probe-restriction enzyme combinations as specified in claim 2 of this application, nor would they occur in clusters. It is surprising and unexpected that different recombinant plants obtained from crossing different *Tripsacum* parents and different *Zea diploperennis* parents carry a high rate of the same mutant alleles. Table 2 on pages 35-46 of the specification describes the novel restriction fragments by molecular weight and probe/enzyme combination and indicates in which recombinant plants each novel fragment is found. Novel restriction fragments are found at 148 out of 176 probed loci. This 84.0% mutation rate is extremely high compared to the expected rates of 0.1 % to 0.0001 %. Perhaps more astounding than the high mutation rate is the fact that different *Tripsacum-diploperennis* recombinants from different parents carry the same mutant allele, i.e. same size restriction fragment. For example, all four *Tripsacum*-teosinte recombinants in Table 2, Sun Dance, 20A, Tripsacorn, and Sun Star, exhibit the same new mutant allele at 39 loci. Such clustering of mutations in plants that are not even sibling progeny from the same parents is unprecedented.

In accordance with the above discussion, it would not be expected by anyone skilled in the art re: claim 6 in Eubanks US Patent No. 5,750,828 1998 that any of the various [(*Tripsacum dactyloides* X *Zea diploperennis)* X *Zea mays*], or [(*Zea diploperennis* X *Tripsacum dactyloides*) X *Zea mays*], or [*Zea mays* X (*Tripsacum dactyloides* X *Zea diploperennis*)], or [Zea mays X (*Zea diploperennis* X *Tripsacum dactyloides*)] hybrid plants described would exhibit the same novel restriction length fragment polymorphisms for each of the respective RFLP probe-restriction enzyme combinations as specified in claim 2 of this application, nor would they occur in clusters. For example, out of the 39 instances in which all four *Tripsacum*-teosinte recombinants carry the same mutant allele, 38 of those mutant alleles were inherited by one or more progeny from crosses between one of the *Tripsacum*-teosinte recombinants and *Zea mays*. This is unprecedented, completely unexpected, and would not have been obvious to anyone skilled in the art.

**Written Description (35 USC § 112)**

The Examiner rejected claims 2-6 and 8-17 under 35 USC § 112 indicating that the claims contain subject matter not described in the specification in such a way to reasonably convey to one skilled in the art that the inventor, at the time the application was filed, had in possession of the claimed invention. The applicant respectfully request reconsideration based on the discussion below, which supports the applicant's possession of the claimed invention in accordance with the guidelines of 35 USC § 112.

The rationale for rejection of claims 2-6 is the specification does not provide adequate description of hybrids between *Tripsacum* and *Zea diploperennis* in terms of their genetic, morphological, and/or physiological characteristics, and it does not provide written description

of genetic, morphological, and/or physiological characteristics of the *Tripsacum* and *Zea diploperennis* parents. The applicant respectfully points out that the specification provides detailed genetic description of the *Tripsacum* and *Zea diploperennis* parents in Table 4, which list the restriction fragments for each probe/enzyme combination by molecular weight that occurs in the *Tripsacum* parents and *Zea diploperennis* parents at 129 nuclear and mitochondrial genetic loci. Likewise, the applicant respectfully points out that the specification provides detailed genetic description of the *Tripsacum-Zea diploperennis* recombinant plants, Sun Dance, 20A, Tripsacorn, and Sun Star, in Tables 2 and 3, which list the restriction fragments for each probe/enzyme combination by molecular weight found at a total of 154 nuclear and mitochondrial genetic loci.

The rationale for rejection of claims 8-12 is the specification does not provide adequate description of hybrids between *Tripsacum* and *Zea diploperennis* in terms of their genetic, morphological, and/or physiological characteristics, and it does not provide written description of genetic, morphological, and/or physiological characteristics of the *Tripsacum* and *Zea diploperennis* parents. The applicant respectfully points out that the specification provides detailed genetic description of the *Tripsacum* and *Zea diploperennis* parents in Table 4, which list the restriction fragments for each probe/enzyme combination by molecular weight found in the *Tripsacum* parents and *Zea diploperennis* parents at 129 nuclear and mitochondrial genetic loci. Likewise, the applicant respectfully points out that the specification provides detailed genetic description of the *Tripsacum-Zea diploperennis* recombinant plants, Sun Dance, 20A, Tripsacorn, and Sun Star, plus the following (*Tripsacum-Zea diploperennis* X maize) recombinant plants, 64SS, 64TC, 2019, 3024, 3028, 3125, 4126, TC64, Sun Devil, 7022, 7024, 9094, 97-5, and V70 in Tables 2 and 3, which list the specific restriction fragments for each

probe/enzyme combination by molecular weight found in each individual plant at 154 nuclear and mitochondrial genetic loci.

The rationale for rejection of claims 13-17 is the specification does not provide adequate description of a maize plant distinguished by the presence of root aerenchyma (claim 13), tolerance to corn rootworm (claim 14), tolerance to drought (claim 15), improved grain quality (claim 16) or tolerance to acid soils (claim 17) in terms of its genetic, morphological, and/or physiological characteristics. The applicant respectfully draws attention in the discussion below to parts of the specification that identify precise maize plants introgressed with genes from *Tripsacum-Zea diploperennis* recombinants that also exhibit the traits claimed in claims 14-17.

Page 24 and pages 27-29 of the specification describe air passages called aerenchyma in *Tripsacum* roots, along with it associated morphological and physiological properties and agronomic traits. Aerenchyma appear in plants for which detailed genetic description is provided in Tables 2 and 3, including the *Tripsacum-Zea diploperennis* recombinants Tripsacorn and Sun Devil, and the recombinant maize plants 7022, 2019, 3028, and TC64. Aerenchyma also appear in *Tripsacum-Zea diploperennis* recombinants Devil Corn and *Zea diploperennis* X *Tripsacum laxum*, plus the recombinant maize plants (7022 X Devil corn), B016 and 6021 derived from TC64, and SDG058 derived from 2019. The presence of aerenchyma allows the plants to withstand droughts as well as flooded soils, and to grow in acid soils. The plants with aerenchyma are distinguished by a novel allele at the locus marked by RFLP probe BNL8.32 and SSR probe bnlg1805. Therefore, evidence is provided in the specification to support claim 13.

As described on pages 24-25, the *Tripsacum-Zea diploperennis* recombinants Tripsacorn, Sun Star, and 20A, and the introgressed maize lines 2019, 3024, 3028, 3125, 4126, TC64, 7022, and 7024, exhibited tolerance to corn rootworm (*Diabrotica vergifera*) in a series of insect

bioassays to select plants resistant to corn rootworm. Detailed genetic descriptions are provided for all of these plants in Tables 2 and 3. By comparison to the genetic profiles of the *Tripsacum-Zea diploperennis-Tripsacum* recombinant Sun Dance that is not resistance, four genetic loci associated with rootworm tolerance were identified. The rootworm tolerant plants are distinguished by a novel allele at the loci marked by RFLP probes BNL5.37, UMC28, UMC103, and UMC95, and SSR probes dupSSR23, phi123, bnlg2235, and bnlg1714. Therefore, evidence is provided in the specification to support claim 14.

As described on page 28 of the specification a progeny population designated SDG058, derived from the recombinant maize plant 2019 for which there is detailed genetic information provided in Tables 2 and 3, was tested to measure drought tolerance in water deficit experiments and compared to experimental maize control W64A. As noted above, SDG058 and plant 2019 are characterized by the presence of aerenchyma in their roots, in contrast to maize which does not have root aerenchyma. Under drought stress the average grain weight of SDG058 was 198 g per plant compared to the average of 125.2 for maize, showing SDG058 has 37% higher yield and is significantly more drought tolerant than maize. Therefore, evidence is provided in the specification to support claim 15.

Since the grain yield of SDG058, which is derived from plant 2019, was 37% higher than maize under drought stress the claim for improved grain quality in claim 16. SDG058 is the progeny of the recombinant maize plant 2019 for which there is detailed genetic information provided in Tables 2 and 3. Descriptions of morphological and physiological traits including presence of root aerenchyma and ability to withstand drought are also provided.

The presence of aerenchyma in *Tripsacum* roots allows the roots to grow deep into the soil below the clay pan. As described on page 27 of the specification, deep subsoils are highly

acidic and *Tripsacum* has the ability to overcome the toxicity associated with acidic soils in which aluminum is solubilized, taken up by the roots, and causes plant injury. Aerenchyma appear in plants for which detailed genetic description is provided in Tables 2 and 3, including the *Tripsacum-Zea diploperennis* recombinants, Tripsacorn and Devil Corn, and recombinant maize plants 7022, 2019, 3028 and TC64. By extension and association, these plants will also be able to tolerate acid soils; thus evidence is provided in the specification to support claim 16.

The detailed genetic descriptions provided in Tables 2 and 3, plus descriptions of morphology, physiology, and experimental results in the specification demonstrate the applicant was in possession of the claimed invention when the application was filed. Therefore, the applicant respectfully requests reconsideration of claims 2-6 and 8-17 under the written description 35 USC § 112.

**Enablement (35 USC § 112)**

The Examiner rejected claims 2-6 and 8-17 under 35 USC § 112, first paragraph, as failing to comply with the enablement requirement. The applicant respectfully requests reconsideration of the enablement rejections based on the discussion below, which addresses the considerations the Examiner raised and shows how the specification provides sufficient guidance to one skilled in the art to be able to make and use the claimed invention.

The rationale for rejection of claims 2-6 is the specification does not provide adequate description of hybrids between *Tripsacum* and *Zea diploperennis* in terms of their genetic, morphological, and/or physiological characteristics, and it does not provide adequate description of genetic, morphological, and/or physiological characteristics of the *Tripsacum* and *Zea diploperennis* parents. The applicant respectfully points out that the specification provides

detailed genetic description in Table 4 of the *Tripsacum* and *Zea diploperennis* parents used to make crosses. Table 4 lists the restriction fragments for each probe/enzyme combination by molecular weight found in the *Tripsacum* parents and *Zea diploperennis* parents at 129 nuclear and mitochondrial genetic loci. The Examiner indicated "there are eight *Tripsacum* plants listed" on page 22 of the specification, yet the specification teaches crosses made with only seven. To clarify, in the specification the count of "seven" included both of the tetraploid *Tripsacum dactyloides* (2n=72) accessions in the same category. The diploid and tetraploid forms of the *Tripsacum dactyloides* are commonly found throughout the eastern, southern and Midwestern United States as a roadside weed. This would be common knowledge to one skilled in the art who could collect their own seed. Seed of *T. dactyloides* can be purchased from a number of seed companies and this would also be known by one skilled in the art. Seeds or clonal material of diploid and tetraploid *T. dactyloides*, *T. laxum* (CEL48770), *T. peruvianum* (DHT-66-13-02), *T. manisurioides* 37553, *T. floridanum* MIA34719, and *Zea diploperennis* accession number 1250 can be obtained through the USDA Germplasm Resources Information Network (available at the GRIN website www.ars-grin/gov). This would be common knowledge to anyone skilled in the art. The appropriate material can be determined by location or accession number provided in the specification and an order for the desired species submitted. For more detailed information about specific *Tripsacum* and *Z. diploperennis* parents and progeny see PP 6,06 7/1989 (Sun Dance), PP 7,977 9/1992 (Tripsacorn), PP 9,640 0/1996 (Sun Star), referenced on pages 8 and 29 of the specification. A sample comprising at least 2500 seeds derived from crosses between *Tripsacum dactyloides* and *Zea diploperennis* as described herein were deposited with American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 on August 2, 1992. The accession number is ATCC75297. A sample of seeds from

crossing *Zea diploperennis* and *Tripsacum laxum* can be deposited with American Type Culture Collection is deemed necessary for this application.

The controlled pollination technique for making crosses between *Tripsacum* and *Z. diploperennis* is described on pages 30-31 of the specification. The RFLP genotyping method is described in the specification on pages 18-21 and the SSR genotyping method is described on pages 17 and 25-26 of the specification. The DNA sequences of the RFLP and SSR probes are publicly available on the Maize Genome Database website (www.maizegdb.org). Anyone skilled in the art would know how to access the probes perform these DNA fingerprinting techniques to determine if plants contained the novel restriction fragments claimed in this application.

The rationale for rejection of claims 8-12 is that the specification does not provide adequate description of hybrids between *Tripsacum* and *Zea diploperennis* in terms of their genetic, morphological, and/or physiological characteristics, and it does not provide adequate description of genetic, morphological, and/or physiological characteristics of the *Tripsacum* and *Zea diploperennis* parents. The applicant respectfully points out that the specification provides detailed genetic description of the *Tripsacum* and *Zea diploperennis* parents in Table 4, which lists the restriction fragments for each probe/enzyme combination by molecular weight found in the *Tripsacum* parents and *Zea diploperennis* parents at 129 nuclear and mitochondrial genetic loci. Likewise, the applicant respectfully points out that the specification provides detailed genetic description of the *Tripsacum-Zea diploperennis* recombinant plants Sun Dance, 20A, Trispacorn and Sun Star, plus the (*Tripsacum-Zea diploperennis* X maize) recombinant plants 64SS, 64TC, 2019, 3024, 3028, 3125, 4126, TC64, Sun Devil, 7022, 7024, 9094, 97-5, and V70 in Tables 2 and 3, which lists the specific restriction fragments for each probe/enzyme

combination by molecular weight found in each individual plant at 154 nuclear and mitochondrial genetic loci. As indicated above, taxa described in the specification and the RFLP probes are publicly available. The plant breeding methods employed to produce and select maize plants introgressed with genes from *Tripsacum-diploperennis* recombinants that are described on pages 3-6 of the specification are common knowledge to one skilled in the art.

The rationale for rejection of claims 13-17 is the specification does not provide adequate guidance regarding the making are use of a maize plant distinguished by the presence of root aerenchyma (claim 13), tolerance to corn rootworm (claim 14), tolerance to drought (claim 15), improved grain quality (claim 16) or tolerance to acid soils (claim 17), nor does it provide adequate description of the genetic, morphological, and/or physiological characteristics of said plants. The applicant respectfully draws attention in the discussion above, under the section Written Description (35 USC § 112), last paragraph of page 11 through first paragraph of page 13, which indicates where in the specification specific maize plants introgressed with genes from *Tripsacum-diploperennis* recombinants that exhibit the traits claimed in claims 13-17 are described. As indicated above, the taxa and the RFLP and SSR probes described in the specification are publicly available. The plant breeding methods employed to produce and select maize plants introgressed with genes from *Tripsacum-diploperennis* recombinants are described on pages 3-6 of the specification. These conventional plant breeding methods would be common knowledge to one skilled in the art.

Referring to Bates et al. (Proceedings of world-wide maize improvement in the 70's and the role of CIMMYT, April 22-26, El Batan, Mexico, 7 pp., 1974) the Examiner pointed out there are sexual barriers to wide hybridizations between *Zea* spp. and *Tripsacum* spp. The Bates et al. report refers to attempts to cross *Zea mays* and *Tripsacum*. Referring to Eubanks

(Economic Botany 49(2): 172-182, 1995), on page 176, first column, line 12 to second column, line 7, Eubanks teaches "previous attempts to cross teosinte and *Tripsacum* have failed to produce viable embryos" (first column, lines 8-10) and that plants derived from crosses between *Tripsacum* and maize were "pollen sterile with limited female fertility" (first column lines 12-15 through second column lines1-4). Page 6 of the specification also describes sexual incompatibility and the difficulty it causes for making crosses **between *Tripsacum* and maize**. Therefore, Eubanks' success in recovering fully fertile recombinants from crossing *Tripsacum* and *Zea diploperennis* was unexpected and would not have been predicted by one skilled in the art. Pages 6-7 of the specification indicate that although a lot of pollinations have to be performed to recover viable seeds, the plants obtained have high fertility, are genetically stable, and are cross-fertile with maize. Consequently, recombinant genes can be easily transferred to maize using conventional plant breeding methods as described in the specification on pages 3-5 and pages 30-31. As indicated above, *Tripsacum* and *Zea diploperennis* germplasm cited in the specification are in the public domain and easily accessible. Pages 30-31 of specification provide the necessary information for making cross pollinations between *Tripsacum* and *Zea diploperennis*. Only time and patience to make a sufficient number of pollinations are needed to recover viable hybrids. No unusual or difficult experimental procedures are required. Once a *Tripsacum-Zea diploperennis* hybrid is established, the plant is perennial and can be maintained indefinitely for making crosses to maize in a marker-assisted, recurrent selection, conventional breeding program. With this clarification, the applicant respectfully requests reconsideration of claims 2-6 and 8-17 under the enablement requirement 35 USC§ 112.

**Claim Rejections (35 USC § 102)**

Claims 2-6 are rejected under 35 USC 102(b) as being anticipated by Eubanks (Theor. Appl. Genet. 94: 707-712, 1997). As stated above and in the specification, basic genetic principles state that the offspring of two parents exhibit a combination of restriction fragments from both parents and can be identified by comparing their DNA fingerprints to those of the parents. As described on page 19 of the specification, hybrid individuals are expected to inherit an allele from each parent at any particular genetic locus. The restriction fragments Eubanks reported in 1997 (see page 708, second column, third paragraph to page 709 first column through second column first paragraph) demonstrated the inheritance of alleles from the *Tripsacum dactyloides* parents and the *Zea diploperennis* parents of $F_1$ *Zea diploperennis* X *Tripsacum dactyloides* and *Tripsacum dactyloides* X *Zea diploperennis* progeny, and it would be expected by anyone skilled in the art that any of the various (*Tripsacum dactyloides* X *Zea diploperennis*) or (*Zea diploperennis* X *Tripsacum dactyloides*) hybrid plants described would exhibit a restriction length fragment polymorphism (RFLP as defined in the specification on page 17, i. e. an allele as defined in the specification page 15) that is the same as in the *Tripsacum dactyloides* parent and an RFLP that is the same as in the *Zea diploperennis* parent for each of the respective RFLP probe-restriction enzyme combinations as specified in claim 2 of this application. This application, however, is distinguished by the surprising and unexpected finding that different recombinant plants obtained from crossing different *Tripsacum* parents and different *Zea diploperennis* parents carry a high rate of the same mutant alleles. Table 2 on pages 35-46 of the specification describes the novel restriction fragments by molecular weight and probe/enzyme combination and indicates in which recombinant plants each novel fragment is found. Novel restriction fragments are found at 148 of the 176 loci probed. Based on these data, the mutation rate is approximately 84.0%. This greatly exceeds the expected mutation rates of 0.1 % to

0.0001 %. Perhaps more astounding is the fact that different *Tripsacum-diploperennis* recombinants from different parents carry the same mutant allele, i.e. same size restriction fragment. For example, all four *Tripsacum-diploperennis* recombinants in Table 2 exhibit the same new mutant allele at 39 loci. Such clustering of mutations is unprecedented. This would never have been expected from the article by Eubanks (Theor. Appl. Genet. 94: 707-712, 1997). The applicant respectfully requests reconsideration of claims 2-6 under 35 USC 102(b) as **not** being anticipated by Eubanks (Theor. Appl. Genet. 94: 707-712, 1997).

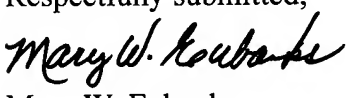
Claims 8-12 and 15 are rejected under 35 USC 102(b) as being anticipated by Eubanks (Plant Patent 7,977, September 15, 1992) and claim 14 is rejected under 35 USC 102(b) as being anticipated by Eubanks (Plant Patent 9,640, September 3, 1996). These patents describe hybrids between *Tripsacum dactyloides* and *Zea diploperennis* that exhibit drought tolerance and tolerance to corn rootworm. The plants in both patents contain many more than one of the restriction fragments in claim 2 (see Table 2, pages 35-36 of the specification). However, for the same reasons given above in regard to the 1997 publication in *Theoretical and Applied Genetics*, this application is distinguished by the surprising and unexpected finding that different recombinant plants obtained from crossing different *Tripsacum* parents and different *Zea diploperennis* parents carry a high rate of the same mutant alleles, and this would never have been anticipated or predicted from the plant patents (Plant Patent 7,977, September 15, 1992 and Plant Patent 9,640, September 3, 1996). The applicant therefore respectfully requests reconsideration of claims 8-12 and 15 under 35 USC 102(b).

In conclusion, based on the discussions presented above, the applicant respectfully requests reconsideration of Claims 2-6 and 8-17 based on the changes to correct wording technicalities in the claims, and discussion in regards to the issues of nonstatutory double

patenting, the written description (35 USC § 113), enablement (35 USC § 113), and claim rejections (35 USC § 102).

Please be advised that the strike-through option is not available on my computer and it was necessary to add new double brackets around double brackets in the claims from the previous communication. Since this gets confusing, a clean copy of the claims is attached for the Examiner's convenience.

Respectfully submitted,



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